Supplementary Materials

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6. **Rescue of PHBA auxotrophy**
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1. General materials and methods

Large-scale optical densities of *E. coli* cultures were determined using cuvettes with a DU 730 Life Sciences UV/Vis spectrophotometer (Beckman Coulter) by measuring absorbance at 600 nm. Growth curves were measured using a BioscreenC (Growth Curves USA). Absorbance at 600 nm was measured every 15 minutes.

Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Varian Inova-500 (500 MHz, 125 MHz). Chemical shifts are reported in parts per million downfield from tetramethylsilane using the solvent resonance as internal standard for ^{1}H (DMSO-*d6* = 2.50 ppm) and ^{13}C (DMSO-*d6* = 39.5 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), and coupling constant.

High Performance Liquid Chromatography (HPLC) traces were recorded using Dionex Ultimate 3000 Rapid Separation LC System. Samples were made using 200 µL of reaction mixture or standard and 200 µL of 0.6% TFA in water (1:1 mixture) in Dionex 1 mL autosampler vials. A Chromolith High Resolution column (RP-18e, 100-4.6 mm, Sorbent Lot/Column Co) was used. Using 0.1% TFA in water (**A**) and 0.1% TFA in acetonitrile (**B**), the standard run consisted of a gradient of 0% to 100% solvent **B** for 25 minutes at a 1 mL/min flow rate. Products were monitored using UV/Vis at 200 nm, 250 nm, 280 nm, 300 nm, and 420 nm. Quantitation of *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, and *o*-hydroxybenzoic acid was accomplished by converting peak areas to concentrations using standard curves generated from known concentrations of authentic standards.

Liquid chromatography-mass spectrometry (LC-MS) analysis was performed in the Small Molecule Mass Spectrometry Facility at the Faculty of Arts and Sciences (FAS) Center for Systems Biology (Harvard University) on an Agilent 1200 series HPLC and 6220 TOF mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) using an electrospray ionization (ESI) source. 5 µL standards and samples were injected onto the HPLC system that consisted of a G1322A vacuum degasser, a G1312B binary pump, a G1367C auto-sampler, a G1316B thermostated column compartment, and a G1315C

DAD, all of which were controlled by the Agilent MassHunter Data Acquisition software version B.04.00. An XTerraTM MS C18 analytical column (2.1x50 mm, 3.5 μm) and a guard column (2.1x10 mm, 3.5 μm) from Waters Corporation (Milford, MA, USA) were used for chromatography, running at a flow rate of 0.4 mL/min and using 0.1% formic acid in water (**A**) and 0.1% formic acid in methanol (**B**) as the solvents.

For analyzing levels of PABA, the following conditions were used: 5% solvent B in solvent A for 2 min, a gradient increasing to 12.3% solvent B in solvent A over 1 min, a gradient increasing to 100% solvent B over 0.8 min, 100% solvent B for 1.8 min, a gradient decreasing to 5% solvent B in solvent A over 1 min, 5% solvent B in solvent A for 1.2 min. The ESI mass spectra data for PABA were recorded on a positive ionization mode for a mass range of m/z 100 to 1200 with reference mass 922.009798 enabled; scan rate, 1 scan/sec; drying gas (N_2) temperature, 350 ºC; drying gas (N_2) flow, 12.0 mL/min; nebulizer, 40 psi; capillary voltage, 3500 V; fragmentor, 150 V; skimmer1, 65.0 V; octopoleRFPeak, 250 V; and elution before 0.8 min and after 6.3 min were diverted to waste. A mass window of ± 0.005Da was used to extract the $[M+H]^+$ ion with a m/z of 138.0550 to create the EIC and calculate peak areas.

For analyzing levels of PHBA, the following conditions were used: 5% solvent B in solvent A for 2 min, a gradient increasing to 27% solvent B in solvent A over 3 min, a gradient increasing to 100% solvent B over 0.8 min, 100% solvent B for 1.8 min, a gradient decreasing to 5% solvent B in solvent A over 0.8 min, 5% solvent B in solvent A for 0.7 min. The ESI mass spectra for PHBA data were recorded on a negative ionization mode for a mass range of m/z 100 to 1200 with reference masses 112.985587 & 1033.988109 enabled; scan rate, 1 scan/sec; drying gas (N_2) temperature, 350 ºC; drying gas (N_2) flow, 12.0 mL/min; nebulizer, 40 psi; capillary voltage, 3500 V; fragmentor, 150 V; skimmer1, 65.0 V; octopoleRFPeak, 250 V; and elution before 0.8 min and after 8 min were diverted to waste. A mass window of ± 0.005Da was used to extract [M-H]- ion with m/z of 137.0244 to create the EIC and calculate peak areas.

All chemicals were obtained from Sigma-Aldrich except for catalyst **2** ([Cp*Ru(cod)Cl]), which was purchased from Strem Chemicals. Solvents were obtained from Sigma-Aldrich except for ethanol (KOPTEC). All NMR solvents were purchased from Cambridge Isotope Laboratories. All water used was purified using a Milli-Q purification system.

2. Strains, media, and culture conditions

Escherichia coli auxotrophs (Δ*pabA* (CGSC Strain #10483), Δ*pabB* (CGSC Strain #9507), and Δ*aroC* (CGSC Strain #9865)) were obtained from the Coli Genetic Stock Center (New Haven, CT). These strains are part of the *E. coli* K-12 Keio Collection (Yamagata, Japan).[1] For routine cultivation all strains were grown aerobically at 37 ºC on M9 glycerol minimal media containing kanamycin (50 μg/mL) that was supplemented with *p*-aminobenzoic acid (PABA) (Δ*pabA* and Δ*pabB* mutants) or with PABA, *p*-hydroxybenzoic acid (PHBA), 2,3,-hydroxybenzoic acid, L-tryptophan, L-phenylalanine, and L-tyrosine (Δ*aroC* mutant).

M9 glycerol minimal media was prepared as follows: 600 mg of Na_2HPO_4, 300 mg of KH_2PO_4, 100 mg of NH_4Cl, and 50 mg of NaCl were dissolved in 90 mL of Milli-Q (MQ) water and autoclaved at 121 ºC for 30 min. Upon cooling to room temperature, the following components were added as sterile solutions: 200 μL of 1.0 M aqueous $MgSO_4$, 10 μL of 1.0 M aqueous $CaCl_2$, and 2.5 mL of aqueous 20% w/v glycerol (0.5% w/v final concentration). For Δ*pabA* and Δ*pabB* mutants, this base media was further supplemented with 100 μL of a 10 mM filter-sterilized solution of PABA. For the Δ*aroC* mutant, base media was further supplemented with 100 μL of 10 mM PABA, 100 μL of 10 mM PHBA, 100 μL of 10 mM 2,3,-hydroxybenzoic acid, 1 mL of 10 mM L-tryptophan, 4 mL of 10 mM L-phenylalanine, and 4 mL of 5 mM L-tyrosine (all solutions were filter sterilized). The final volume was brought to 100 mL using autoclaved Milli-Q water. All media were stored at 4 ºC.

To prepare PABA-free M9 glycerol minimal media for PABA auxotroph rescue experiments, M9 glycerol base media was prepared as above for Δ*pabA*/Δ*pabB* and Δ*aroC* mutants and was supplemented with all of the required nutrients except PABA.

Reaction components were added to PABA-free M9 glycerol media as noted in the description of individual experiments. To prepare PHBA-free M9 glycerol minimal media for PHBA auxotroph rescue experiments, M9 glycerol base media was prepared as above for the Δ*aroC* mutant and was supplemented with all of the required nutrients except PHBA. Reaction components were added to PHBA-free M9 glycerol media as detailed in the description of individual experiments.

All endpoint rescue experiments were performed in 5 mL of media in sterile 17 x 100 mm Round-Bottom Disposable Capped Polystyrene Culture Tubes (BD Falcon, product #60819-524). Culture tubes were incubated at 37 ºC on a rotating disk (60 rpm). Growth curve rescue experiments were performed in 150 μL of media in sterile 100 well Honeycomb 2 microplates (Growth Curves USA, product #9502550) at 37 ºC with continuous shaking (120 rpm).

The growing *E. coli* cultures used for rescue experiments were inoculated from 5 mL overnight M9 glycerol cultures on the same day of the reaction. The overnight cultures were inoculated the morning before from frozen glycerol stocks and were incubated aerobically at 37 ºC for 48 h (to an $OD_{600} > 1.0$). Each overnight culture was then diluted $1{:}10^5$ into the appropriate PABA-free or PHBA-free M9 glycerol minimal media containing kanamycin (50 μg/mL) prior to inoculation. This dilution factor was sufficient to bring the concentration of residual PABA or PHBA (< 0.1 nM) in the inoculum below that necessary to support growth (1-10 nM).

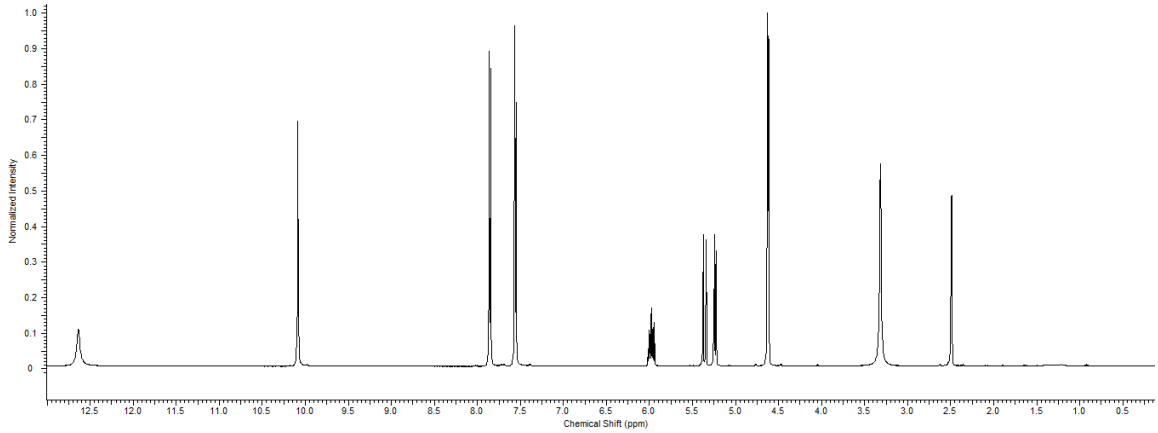
3. Synthesis of Alloc-PABA (1)

4-(Allyloxycarbonylamino)benzoic acid (**1**) was synthesized according to a previously reported procedure.[2] To a stirred solution of PABA (1.00 g, 7.29 mmol) in H_2O/dioxane (10 mL/10 mL) at room temperature was added *N,N*-diisopropylethylamine (2.60 mL, 14.9 mmol) and $NaHCO_3$ (1.80 g, 21.8 mmol). Allyl chloroformate (930 μL, 8.74 mmol) was added dropwise to the reaction mixture and the resulting solution was stirred overnight. The reaction mixture was quenched by addition of 1 M aqueous hydrochloric

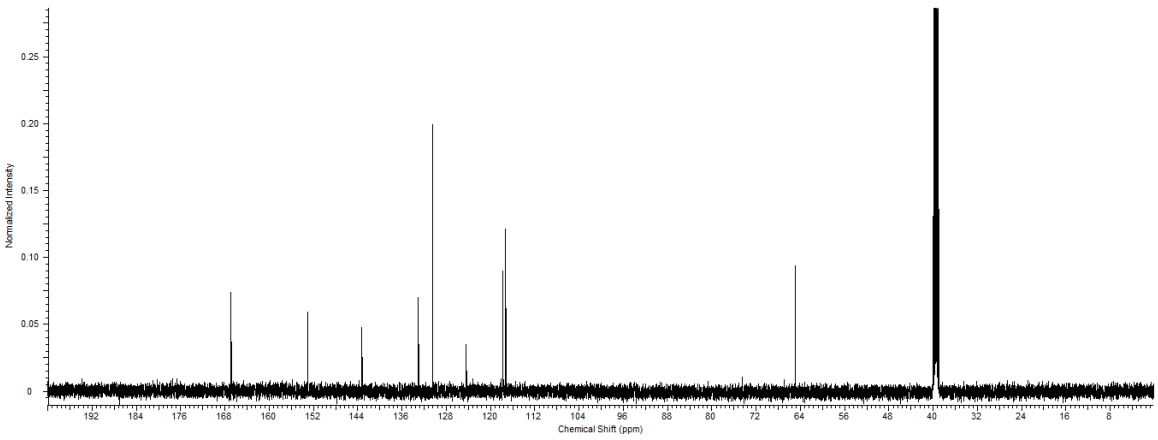
acid (90 mL). Once gas evolution stopped, the solid residue was collected by filtration and dried overnight under high vacuum. This material was purified using flash column chromatography on silica gel (10% acetonitrile in dichloromethane) to give 4-(allyloxycarbonylamino)benzoic acid (**1**) (1.35 g, 84%). Characterization data matched that reported previously.[2]

^{1}H NMR (500 MHz, DMSO-d_6): δ 12.64 (broad s, 1H, CO_2**H**), 10.09 (broad s, 1H, N**H**), 7.85 (dd, 2H, J = 8.5, 1.1 Hz, Ar**H**), 7.56 (dd, 2H, J = 8.7, 1.5 Hz, Ar**H**), 5.97 (ddd, 1H, J = 17.0, 10.1, 1.5 Hz, C**H**=CH_2), 5.36 (dd, 1H, J = 17.1, 1.8 Hz, CH=C**H**$_2$), 5.24 (d, 1H, J = 10.1 Hz, CH=C**H**$_2$), 4.62 (dd, 2H, J = 5.5, 1.4 Hz, C**H**$_2$CH=CH_2).
^{13}C NMR (125 MHz, DMSO-d_6): δ 166.92, 153.00, 143.27, 133.03, 130.42, 124.37, 117.79, 117.31, 64.90.

^{1}H NMR:



^{13}C NMR:



4. In vitro deprotection of 1 with [Cp*Ru(cod)Cl] (2)

In vitro deprotection experiments were performed in 5 mL of PABA-free M9 glycerol minimal media containing kanamycin (50 µg/mL) in sterile 17 x 100 mm Round-Bottom Disposable Capped Polystyrene Culture Tubes.

Reactions with 100 µM substrate **1**: Substrate **1** (50 µL of a 10 mM solution in PABA-free M9 glycerol minimal media, 100 µM, 1.0 equiv) and catalyst **2** (20 µL of a 5 mM solution in DMSO, 20 µM, 0.2 equiv) were added sequentially to the reaction mixture followed by thiophenol or glutathione (50 µL of a 50 mM aqueous solution, 500 µM, 5.0 equiv). The reaction mixture was incubated on a rotating disk (60 rpm) at 37 ºC. For reactions run in the absence of catalyst, 20 µL of DMSO was added to the reaction mixture. After 48 hours, a 200 µL aliquot of the reaction mixture was removed and quenched with 200 µL of 0.6% aqueous trifluoroacetic acid. 90 µL of the quenched reaction mixture was analyzed using HPLC as described in the 'General materials and methods' section.

Figure S1: HPLC traces of in vitro deprotection reactions run with 100 µM substrate **1**.

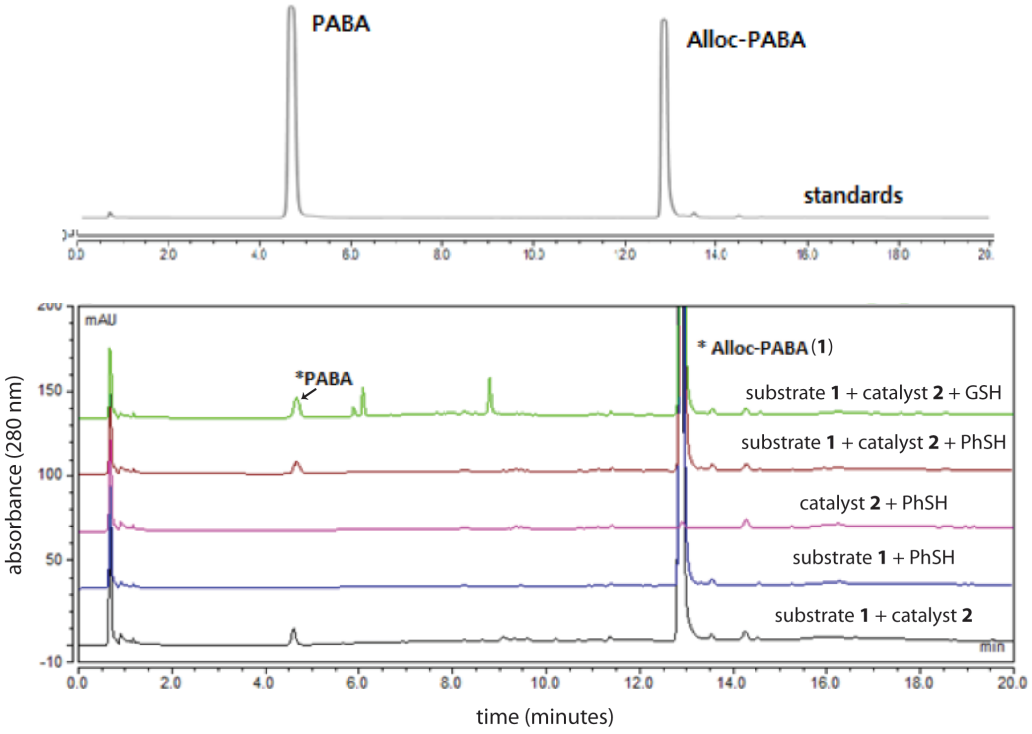


Table S1: Quantification of PABA generated in deprotection reactions run with 100 µM substrate **1** from HPLC peak areas.

Reaction	Area	Concentration of PABA (µM)	Conversion (%)
Substrate **1** + catalyst **2**	0.9742	8.22	8.22
Substrate **1** + Catalyst **2** + PhSH	1.0801	9.08	9.08
Substrate **1** + Catalyst **2** + GSH	1.6745	13.9	13.9

Reactions with 10 µM substrate **1**: Substrate **1** (5 µL of a 10 mM solution in PABA-free M9 glycerol minimal media, 10 µM, 1.0 equiv) and catalyst **2** (2 µL of a 5 mM solution in DMSO, 2 µM, 0.2 equiv) were added sequentially to the reaction mixture followed by thiophenol or glutathione (5 µL of a 50 mM aqueous solution, 50 µM, 5.0 equiv). The reaction mixture was incubated on a rotating disk (60 rpm) at 37 ºC. For reactions run in the absence of catalyst, 2 µL of DMSO was added to the reaction mixture. After 48 hours, a 200 µL aliquot of the reaction mixture was removed and quenched with 200 µL of 0.6% aqueous trifluoroacetic acid. 90 µL of the quenched reaction mixture was analyzed using HPLC as described in the 'General materials and methods' section.

Figure S2: HPLC traces of in vitro deprotection reactions run with 10 µM substrate **1**.

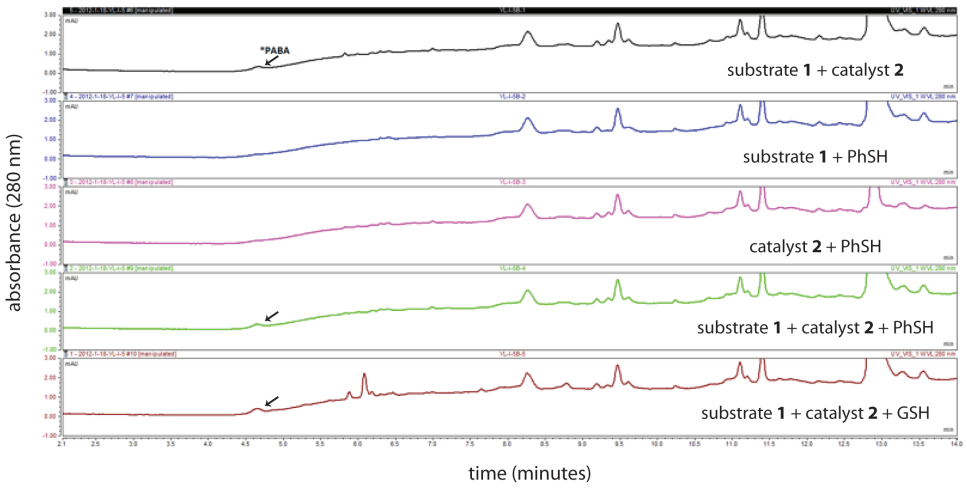


Table S2: Quantification of PABA generated in deprotection reactions run with 10 μM substrate **1** from HPLC peak areas.

Reaction	Area	Concentration of PABA (μM)	Conversion (%)
Substrate **1** + catalyst **2**	0.0108	0.09	0.9
Substrate **1** + Catalyst **2** + PhSH	0.0119	0.10	1.0
Substrate **1** + Catalyst **2** + GSH	0.0230	0.20	2.0

5. Rescue of PABA auxotrophy

Endpoint rescue experiments and control reactions

To the appropriate PABA-free M9 glycerol minimal media containing kanamycin (50 μg/mL) was added substrate **1** (5 μL of a filter sterilized 10 mM solution in PABA-free M9 glycerol minimal media, 10 μM, 1.0 equiv), diluted Δ*pabA*, Δ*pabB*, or Δ*aroC* overnight culture (10 μL, prepared as described in the 'Strains, media, and culture conditions' section), and catalyst **2** (10 μL of a 1 mM solution in DMSO, 2 μM, 0.2 equiv) to give a final volume of 5 mL. These rescue experiments were performed alongside a positive control (inoculation into PABA-free M9 glycerol minimal media containing 10 μM PABA and 10 μL DMSO) and two negative controls (inoculation into PABA-free M9 glycerol minimal media containing 10 μL DMSO and inoculation into PABA-free M9 glycerol minimal media containing 10 μM substrate **1** and 10 μL DMSO). Each culture was grown at 37 °C for 48 h before measuring OD_{600}.

A more extensive set of control experiments was carried out in an analogous manner for all three mutants in the appropriate PABA-free M9 glycerol minimal media containing kanamycin (50 μg/mL) (Table S3). When applicable, thiol additive (thiophenol or reduced L-glutathione) was added last as a filter-sterilized aqueous solution (5 μL of a 50 mM solution, 50 μM, 5.0 equiv). Cultures were grown at 37 °C for 48 h before measuring OD_{600}.

Table S3: Impact of rescue reaction conditions on the growth of PABA auxotrophs.

Entry	Conditions	OD_{600} (Δ*pabA*)	OD_{600} (Δ*pabB*)	OD_{600} (Δ*aroC*)
1	PABA-free M9 glycerol minimal media + DMSO	0.000	0.000	0.000
2	PABA-free M9 glycerol minimal media + 10 μM PABA + DMSO	1.334	1.275	1.103
3	PABA-free M9 glycerol minimal media + 10 μM **1** + DMSO	0.000	0.000	0.002
4	PABA-free M9 glycerol minimal media + 2 μM **2** + DMSO	0.000	0.000	0.000
5	PABA-free M9 glycerol minimal media + 50 μM PhSH + DMSO	0.000	0.000	0.000
6	PABA-free M9 glycerol minimal media + 10 μM **1** + 50 μM PhSH + DMSO	0.000	0.000	0.002
7	PABA-free M9 glycerol minimal media + 2 μM **2** + 50 μM PhSH	0.000	0.000	0.000
8	PABA-free M9 glycerol minimal media + 10 μM **1** + 2 μM **2**	1.106	1.415	1.133
9	PABA-free M9 glycerol minimal media + 10 μM **1** + 2 μM **2** + 50 μM PhSH	1.177	1.419	1.168
10	PABA-free M9 glycerol minimal media + 10 μM **1** + 2 μM **2** + 50 μM GSH	1.358	1.397	1.179

Growth curves

Small scale cultures for growth curve analysis were prepared in triplicate. To the appropriate PABA-free M9 glycerol minimal media was added kanamycin (3 μL, 50 μg/mL), substrate **1** (3 μL of a filter-sterilized 0.5 mM solution in PABA-free M9 glycerol minimal media, 10 μM, 1 equiv), diluted Δ*pabA*, Δ*pabB*, or Δ*aroC* overnight culture (10 μL, overnight culture was diluted 2 x 10^7-fold into the appropriate PABA-free M9-glycerol minimal media containing kanamycin), and catalyst **2** (3 μL of a 100 μM solution in DMSO, 2 μM, 0.2 equiv) to give a final culture volume of 150 μL. These rescue experiments were performed alongside a positive control (inoculation into PABA-free M9 glycerol minimal media containing 10 μM PABA and 3 μL DMSO) and two negative controls (inoculation into PABA-free M9 glycerol minimal media containing 3 μL DMSO and inoculation into PABA-free M9 glycerol minimal media containing 10 μM substrate **1** and 3 μL DMSO). The cultures were incubated at 37 ºC in the Bioscreen C as described in the 'Strains, media, and culture conditions' section. OD600

measurements were collected every 15 min over 72 h. OD_{600} measurements from the triplicate cultures were averaged to generate the final growth curves.

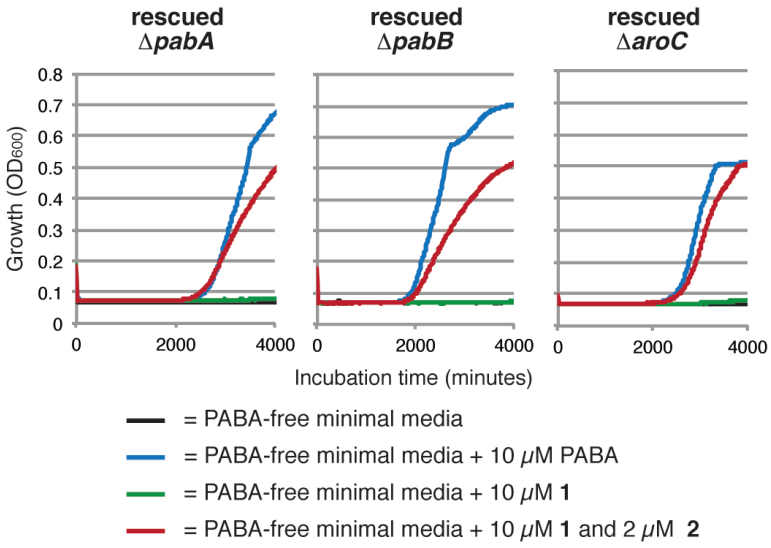
Experiments with rescued strains

To prepare inocula for these experiments, rescue experiments were performed as described above for each of the mutants in 5 mL of the appropriate M9 glycerol minimal media containing substrate **1** (10 μM) and catalyst **2** (2 μM). The cultures were grown at 37 ºC for 48 h and reached the following optical densities: Δ*pabA* = 1.106, Δ*pabB* = 1.373, and Δ*aroC* = 1.280. Each rescued culture was diluted 10^5-fold into PABA-free M9 glycerol minimal media as described in the 'Strains, media, and culture conditions' section. The diluted cultures were used to inoculate both 5 mL cultures for endpoint rescue experiments and triplicate 150 μL cultures for growth curve analysis as described above. Both series of experiments included a rescue experiment (PABA-free M9 glycerol minimal media containing both substrate **1** (10 μM) and catalyst **2** (2 μM)), a positive control (PABA-free M9 glycerol minimal media containing 10 μM PABA), and two negative controls (PABA-free M9 glycerol minimal media with DMSO and PABA-free M9 glycerol minimal media containing 10 μM substrate **1** and DMSO).

Table S4: Impact of rescue reaction conditions on the growth of previously rescued PABA auxotrophs (5 mL culture volume).

Entry	Conditions	OD_{600} (Δ*pabA*)	OD_{600} (Δ*pabB*)	OD_{600} (Δ*aroC*)
1	PABA-free M9-glycerol minimal media + DMSO	0.000	0.000	0.001
2	PABA-free M9-glycerol minimal media + 10 μM PABA + DMSO	1.308	1.259	1.319
3	PABA-free M9-glycerol minimal media + 10 μM **1** + DMSO	0.004	0.008	0.002
4	PABA-free M9-glycerol minimal media + 10 μM **1** + 2 μM **2**	1.343	1.388	1.329
5	PABA-free M9-glycerol minimal media + 10 μM **1** + 2 μM **2** + 50 μM PhSH	1.285	1.339	1.301
6	PABA-free M9-glycerol minimal media + 10 μM **1** + 2 μM **2** + 50 μM GSH	1.384	1.457	1.196

Figure S3: Growth curves for reinoculation of previously rescued PABA auxotrophs. Curves shown are the average of three replicate cultures.



Detection of PABA in rescued cultures

The minimum concentration of PABA needed to support the growth of Δ*pabA* and Δ*aroC* mutants in M9 glycerol minimal media was determined by evaluating a series of cultures containing varying amounts of PABA. To the appropriate PABA-free M9 glycerol minimal media containing kanamycin (50 μg/mL) was added PABA (5 μL of an appropriate filter-sterilized dilution in PABA-free M9 glycerol minimal media to give 100 nM, 10 nM, 1 nM, 0.1 nM, 0.01 nM, or 0.001 nM final PABA concentration) and diluted Δ*pabA* or Δ*aroC* overnight culture (10 μL, prepared as described in the 'Strains, media, and culture conditions' section) to give a final volume of 5 mL. The cultures were incubated at 37 °C for 48 h before measuring OD_{600}.

Table S5: Impact of PABA concentration on the growth of Δ*pabA* and Δ*aroC* mutants.

Final concentration of PABA in M9 glycerol minimal medium	OD_{600} (Δ*pabA*)	OD_{600} (Δ*aroC*)
100 nM	1.054	1.331
10 nM	0.462	0.566
1 nM	0.001	0.004
0.1 nM	0.000	0.001
0.01 nM	0.000	0.001
0.001 nM	0.000	0.000

Detection and quantitation of PABA in the spent media of a rescued Δ*pabA* mutant cultures was carried out using LC-MS. A standard curve was generated using PABA stock solutions of various concentrations. Two 5 mL rescue experiments containing substrate **1** (10 or 100 μM), the Δ*pabA* mutant, and catalyst **2** (2 or 20 μM) were performed as described above. A negative control containing 100 μM substrate **1** and the Δ*pabA* mutant was also run alongside the rescue experiments. After 48 h of incubation at 37 ºC, a 500 μL aliquot of each culture was removed, centrifuged (20 min x 3000 rpm), and 200 μL of the supernatant was quenched with 200 μL of 0.6% aqueous trifluoroacetic acid. This sample was analyzed using LC-MS as described in the 'General materials and methods section'.

Table S6: Detection of PABA in media from rescued cells using LC-MS.

Reaction	Observed Mass	Error (ppm)	Area	Concentration of PABA (μM)
100 μM Substrate **1** + 20 μM catalyst **2**	138.0550	-0.38	1286223	4.3
10 μM Substrate **1** + 2 μM catalyst **2**	Not detected[a]	Not detected	15642	0.06

[a] A peak with a retention time matching that of a PABA standard solution was found in the EIC; however, the low signal level did not allow the PABA mass to be observed.

6. Rescue of PHBA auxotrophy

Endpoint rescue experiments and control reactions

To PHBA-free M9 glycerol minimal media containing kanamycin (50 μg/mL) was added benzoic acid (**3**) (50 or 100 μL of a filter sterilized 10 mM solution in PHBA-free M9 glycerol minimal media, 100 or 200 μM, 1.0 equiv), diluted Δ*aroC* overnight culture (10 μL, prepared as described in the 'Strains, media, and culture conditions' section), citric acid (20 or 40 μL of a filter sterilized 500 mM solution in PHBA-free M9 glycerol minimal media, 2 or 4 mM, 20 equiv), and $FeSO_4$ (100 or 200 μL of a filter sterilized 5 mM aqueous solution, 100 or 200 μM, 1.0 equiv) to give a final volume of 5 mL. These

rescue experiments were performed alongside a positive control (inoculation into PHBA-free M9 glycerol minimal media containing 100 μM PHBA) and two negative controls (inoculation into PHBA-free M9 glycerol minimal media and inoculation into PHBA-free M9 glycerol minimal media containing 100 μM **3**). Each culture was grown at 37 °C for 48 h before measuring OD_{600}. A more extensive set of control reactions was carried out in an analogous manner in PHBA-free M9 glycerol minimal media containing kanamycin (50 μg/mL) (Table S7). Cultures were grown at 37 °C for 48 h before measuring OD_{600}.

Table S7: Impact of rescue reaction conditions on the growth of PHBA auxotrophs.

Entry	Conditions	OD_{600} (Δ*aroC*)
1	PHBA-free M9 glycerol minimal media	0.000
2	PHBA-free M9 glycerol minimal media + 100 μM PHBA	1.425
3	PHBA-free M9 glycerol minimal media + 100 μM **3**	0.000
4	PHBA-free M9 glycerol minimal media + 2 mM citric acid	0.000
5	PHBA-free M9 glycerol minimal media + 100 μM $FeSO_4$	0.000
6	PHBA-free M9 glycerol minimal media + 100 μM **3** + 2 mM citric acid	0.000
7	PHBA-free M9 glycerol minimal media + 100 μM **3** + 100 μM $FeSO_4$	0.011
8	PHBA-free M9 glycerol minimal media + 2 mM citric acid + 100 μM $FeSO_4$	0.000
9	PHBA-free M9 glycerol minimal media + 10 μM **3** + 200 μM citric acid + 10 μM $FeSO_4$	0.001
10	PHBA-free M9 glycerol minimal media + 100 μM **3** + 2 mM citric acid + 100 μM $FeSO_4$	0.944
11	PHBA-free M9 glycerol minimal media + 200 μM **3** + 4 mM citric acid + 200 μM $FeSO_4$	1.634

Growth curves

Small scale cultures for growth curve analysis were prepared in triplicate. To PHBA-free M9 glycerol minimal media was added kanamycin (3 μL, 50 μg/mL), benzoic acid (**3**) (10 μL of a filter-sterilized 3 mM solution in PHBA-free M9 glycerol minimal media, 200 μM, 1 equiv), diluted Δ*aroC* overnight culture (10 μL, overnight culture was then diluted 2 x 10^7-fold into the appropriate PHBA-free M9-glycerol minimal media

containing kanamycin), citric acid (10 μL of a filter sterilized 60 mM solution in PHBA-free M9 glycerol minimal media, 4 mM, 20 equiv), and $FeSO_4$ (10 μL of a filter sterilized 3 mM aqueous solution, 200 μM, 1.0 equiv) to give a final culture volume of 150 μL. These rescue experiments were performed alongside a positive control (inoculation into PHBA-free M9 glycerol minimal media containing 200 μM PHBA) and two negative controls (inoculation into PHBA-free M9 glycerol minimal media and inoculation into PHBA-free M9 glycerol minimal media containing 200 μM **3**). The cultures were incubated at 37 ºC in the Bioscreen C as described in the 'Strains, media, and culture conditions' section. OD_{600} measurements were collected every 15 min over 72 h. Measurements from the triplicate cultures were averaged to generate the final growth curves.

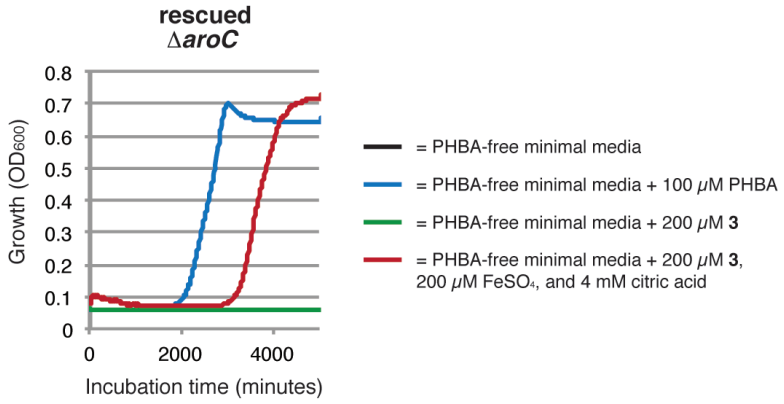
Experiments with rescued strains

To prepare inocula for these experiments, a rescue experiment was performed as described above for the Δ*aroC* mutant in 2.5 mL of PHBA-free M9 glycerol minimal media containing benzoic acid (**3**) (100 μM), citric acid (2 mM), and $FeSO_4$ (100 μM). The culture was grown at 37 ºC for 60 h and reached an OD_{600} of 1.664. The rescued culture was diluted 10^5-fold into PHBA-free M9 glycerol minimal media as described in the 'Strains, media, and culture conditions' section. The diluted cultures were used to inoculate both 5 mL cultures for endpoint rescue experiments and triplicate 150 μL cultures for growth curve analysis as described above. Both series of cultures included a rescue experiment (PHBA-free M9 glycerol minimal media containing **3** (100 or 200 μM), citric acid (2 or 4 mM), and $FeSO_4$ (100 or 200 μM)), a positive control (PHBA-free M9 glycerol minimal media containing 100 μM PHBA), and two negative controls (PHBA-free M9 glycerol minimal media and PHBA-free M9 glycerol minimal media containing 100 μM substrate **3**).

Table S8: Impact of rescue reaction conditions on the growth of previously rescued PHBA auxotrophs (5 mL culture volume).

Entry	Conditions	OD_{600} (Δ*aroC*)
1	PHBA-free M9-glycerol minimal media	0.000
2	PHBA-free M9-glycerol minimal media + 100 μM PHBA	0.914
3	PHBA-free M9-glycerol minimal media + 100 μM **3**	0.000
4	PHBA-free M9-glycerol minimal media + 2 mM citric acid	0.000
5	PHBA-free M9-glycerol minimal media + 100 μM $FeSO_4$	0.000
6	PHBA-free M9-glycerol minimal media + 100 μM **3** + 2 mM citric acid	0.000
7	PHBA-free M9-glycerol minimal media + 100 μM **3** + 100 μM $FeSO_4$	0.000
8	PHBA-free M9-glycerol minimal media + 2 mM citric acid + 100 μM $FeSO_4$	0.000
9	PHBA-free M9-glycerol minimal media + 100 μM **3** + 2 mM citric acid + 100 μM $FeSO_4$	0.928
10	PHBA-free M9-glycerol minimal media + 200 μM **3** + 4 mM citric acid + 200 μM $FeSO_4$	1.659

Figure S4: Growth curves for reinoculation of rescued Δ*aroC* mutant. Curves shown are the average of three replicate cultures.



Detection of PHBA in rescued cultures

The minimum concentration of PHBA needed to support the growth of the Δ*aroC* mutant in M9 glycerol minimal media was determined by evaluating a series of cultures containing varying amounts of PHBA. To the appropriate PHBA-free M9 glycerol

minimal media containing kanamycin (50 μg/mL) was added PHBA (2.5 μL of an appropriate filter-sterilized dilution in PHBA-free M9 glycerol minimal media to give 10 μM, 1μM, 100 nM, 10 nM, 1 nM, 0.1 nM, or 0.01 nM final PHBA concentration) and diluted Δ*aroC* overnight culture (10 μL, prepared as described in the 'Strains, media, and culture conditions' section) to give a final volume of 2.5 mL. The cultures were incubated at 37 ºC for 48 h before measuring OD_{600}.

Table S9: Impact of PHBA concentration on the growth of the Δ*aroC* mutant.

Final concentration of PHBA in M9 glycerol minimal medium	OD_{600} (Δ*aroC*)
10 μM	1.077
1 μM	1.255
100 nM	1.377
10 nM	0.012
1 nM	0.000
0.1 nM	0.000
0.01 nM	0.000

Detection and quantitation of PHBA in the spent media of rescued Δ*aroC* mutant cultures was carried out using LC-MS. A standard curve was generated using PHBA stock solutions of various concentrations. Two 5 mL rescue experiments with benzoic acid (**3**) (100 or 200 μM), the Δ*aroC* mutant, citric acid (2 or 4 mM), and $FeSO_4$ (100 or 200 μM) were performed as described above. A negative control containing 100 μM substrate **3** and the Δ*aroC* mutant was also run alongside the rescue experiments. After 54 h of incubation at 37 ºC, a 500 μL aliquot of each culture was removed, centrifuged (20 min x 3000 rpm), and 200 μL of the supernatant was quenched with 200 μL of 0.6% aqueous trifluoroacetic acid. The samples were analyzed using LC-MS as described in the 'General materials and methods section'.

Table S10: Detection of PHBA in media from rescued cells using LC-MS.

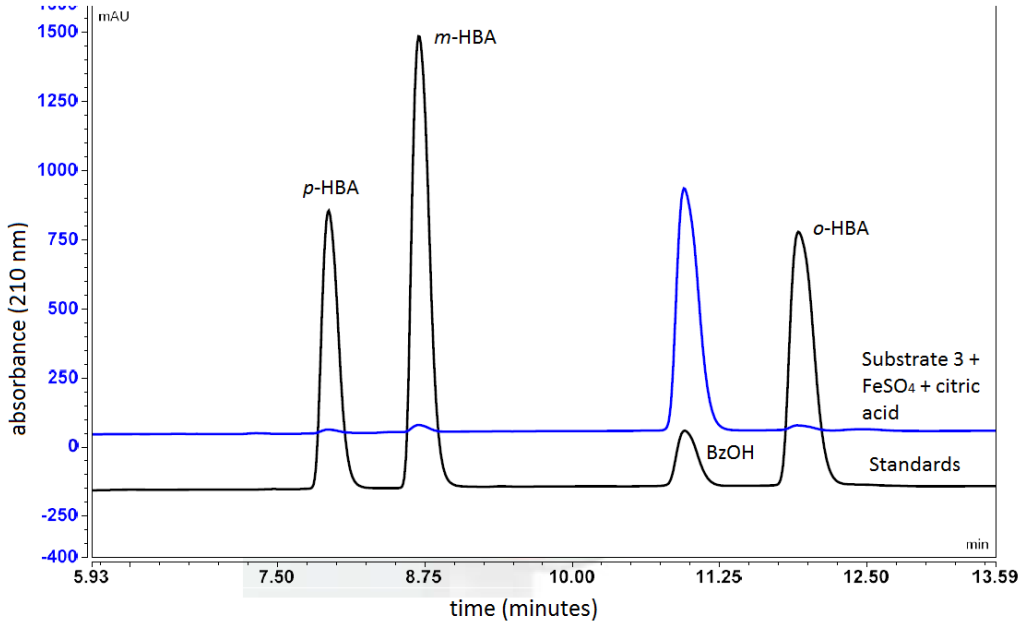
Reaction[a]	Observed Mass	Error (ppm)[a]	Area	Concentration of PHBA (μM)
100 μM **3** + 2 mM citric acid + 100 μM $FeSO_4$	137.0255	-8.18	60741	0.07
200 μM **3** + 4 mM citric acid + 200 μM $FeSO_4$	137.0255	-7.78	74868	0.09

[a] A peak in the EIC matched the retention time of a PHBA standard solution.

Determination of product distribution

To assess the regioselectivity of the iron-catalyzed hydroxylation of benzoic acid under the conditions of rescue, we carried out an in vitro reaction in PHBA-free M9 glycerol minimal media using increased concentrations of reagents. Benzoic acid (**3**) (250 μL of a 10 mM solution in filter-sterilized PHBA-free M9 glycerol minimal media, 1 mM, 1.0 equiv), citric acid (100 μL of a filter sterilized 500 mM solution in PHBA-free M9 glycerol minimal media, 20 mM, 20 equiv), and $FeSO_4$ (50 μL of a filter sterilized 50 mM aqueous solution, 1 mM, 1.0 equiv) were added sequentially to PHBA-free M9 glycerol minimal media to give a total volume of 2.5 mL. The reaction mixture was incubated on a rotating disk (60 rpm) at 37 ºC. After 24 hours, a 200 μL aliquot of the reaction mixture was removed and quenched with 200 μL of 0.6% aqueous trifluoroacetic acid. 90 μL of the quenched reaction mixture was analyzed using HPLC as described in the 'General materials and methods' section except that a flow rate of 0.7 mL/min was used. The concentrations of the three isomeric monohydroxylation products were determined by integration and comparison of peak area with standard curves prepared using authentic standards.

Figure S5: HPLC traces of in vitro iron-mediated hydroxylation of 1 mM substrate **3**.



BzOH = benzoic acid, HBA = hydroxybenzoic acid.

Table S11: Quantification of *o*, *m*, *p*-hydroxybenzoic acid generated in iron-mediated hydroxylation reaction with 1 mM substrate **3** from HPLC peak areas.

Product	Area	Concentration of product (µM)	Conversion (%)
o-hydroxybenzoic acid (*o*-HBA)	3.6005	3.24	0.324
m-hydroxybenzoic acid (*m*-HBA)	4.0562	2.90	0.29
p-hydroxybenzoic acid (*p*-HBA)	2.2991	3.14	0.314

References

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